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| 13. ABSTRACT (Maximum 200 Words) Functional redundancy in Base Excision repair (BER) may permit polymorphism to accumulate in these parallel pathways. Deficiencies in BER may lead to elevated spontaneous mutation rates and an earlier onset of cancer. We have analysed two BER enzymes: TDG and MED1(MBD4), both DNA N-glycosylases that remove the T residue in a T/G mismatch and the U residue in a U/G mismatch. Our analysis of 100 cancer patients revealed only one common polymorphism and no rare polymorphisms for MED1. MED1 polymorphisms are random and appear not to be related to cancer. The TDG gene revealed two common polymorphisms, in exon 5 and exon 10, and no rare polymorphisms, in our analysis of 268 patients. Curiously, we observed no patient that harbors both polymorphisms when eight such patients were expected based on the allelic frequency of these polymorphisms. If this observation holds true for a larger population study, it may imply that the TDG gene has a second role in humans besides DNA repair, and that the presence of both alleles in a person may lead to embryonic lethality. The OGG1 gene and APE1 gene will be examined next to test for linkage to the polymorphisms in the TDG gene. | | | | |
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Base Excision Repair Gene Mutations and Polymorphisms as a Potential Modifier of Breast Cancer Risk

INTRODUCTION

The focus of this research is to evaluate the possibility that DNA polymorphisms altering the amino acid sequences of the enzymes of base excision repair (BER) may be related to cancer. BER is one of three major ubiquitous DNA repair systems protecting all cells. Lesions repaired by BER include the oxidation of DNA by oxygen, and the result of the natural instability of the bases of DNA, leading to the deamination of cytosine and 5-methylcytosine, and the opening of the ring structures in DNA bases. We focus on BER systems for two important reasons. First, unlike carcinogens, the DNA lesions repaired by BER occur spontaneously in each cell thousands of times per day such that the pressure towards mutations is always present. If BER were compromised, a cascade of gene damages may lead to cancer. Secondly, the cell uses two BER enzymes against each spontaneous lesion to provide extra safety. Our hypothesis is that this functional redundancy in BER permits the opportunity for mutations and polymorphism to accumulate in these parallel pathways. Sporadic cancers may be associated with germline defects in different genes of BER. Because there are many BER enzymes, the association of each one with breast cancer may appear to be sporadic instead of familial. Having two undesirable BER polymorphisms/mutations in the same person would be much worst than having just one.

We proposed to examine four BER genes, MED 1 and TDG that are the first enzymes in the pathways for detecting and repairing cytosine deamination reactions, and OGG1 and hMYH that are for repairing the damage and mutations produced by the oxidative damage lesion 8-oxoguanine.

We proposed to include two hundreds disease-free women as our reference control group. The three test groups will consist of (i) two hundred non-BRCA1/2 women (or maximum number available) with sporadic breast cancer, (ii) two hundred (or maximum number available) BRCA1/2 women without cancer, and (iii) two hundred (or maximum number available) BRCA1/2 women with breast cancer. We will screen their germline DNA for the presence of BER gene defects in the coding regions, and to test for the correlation of these BER gene defects with breast cancer in the presence or absence of BRCA1/2 mutations.

BODY

The following report describes the progress with respect to the area of investigation described in my original proposal. Because it is a Concept award, a one page proposal, the anticipated tasks were not listed in the proposal:

Task 1: Months 7-12 Screening 100 research participants for polymorphisms in MED 1

We developed PCR primers and optimized the CEL I mutation detection assay for screening the MED 1 gene. The MED 1 gene has eight exons and each was screened as one PCR product except exon 3 that was divided into two overlapping PCR products of about 500 bp each. The method developed in this laboratory to detect unknown polymorphisms is called CEL I mismatch detection (1,-3). CEL I, from celery, is representative of a family of mismatch-specific endonucleases found in plants. PCR primers of two fluorescent colors are used to PCR to amplify an exon of a chosen gene of a research participant. When the PCR product is denatured by heat and then renatured, heteroduplexes will be formed in a fraction of the DNA. CEL I cuts these heteroduplexes at the 3' side of the mismatch, in one DNA strand per duplex. Because two different mismatches can form from the PCR products, CEL I can cut the mismatch in either the top strand or the bottom strand at a mutation, and produces one truncated band for each color. The sizes of the bands are measured by GeneScan software, with the help of internal standards in each lane, in the ABI automated DNA sequencer. The sum of the products of the two colors should be one nucleotide longer than the full-length PCR product.

The panel of DNA we were able to obtain immediately to start the study were from the peripheral blood lymphocytes of patients diagnosed with ovarian cancer. Although this award proposes to examine breast cancer, because breast cancer and ovarian cancer and genetically associated, we decided to use this ovarian cancer panel for our initial screen and practice. We analyzed the exons of all 100 patients twice, initially as pools of three patients in one reaction, so as to detect homozygous polymorphisms. Subsequently, we analyzed one individual per reaction, because we could not find any rare polymorphisms other than a single frequently mutation in exon 3 that changes an alanine residue to a threonine residue.

Task 2: Months 1-6 Cloning and sequencing to confirm the genomic sequences of the TDG gene.

The sequence of the TDG gene was not in complete agreement in literature at the start of this project. Published intron sequence information was also inadequate for the planning of good PCR primers. We therefore undertook the cloning and sequencing of part of the TDG gene to obtain exon and intron information to design better PCR primers for mutation detection. We confirmed one published finding that the TDG gene has 10 exons and not 8 exons as reported by another finding. As will be shown below, our mutation screening using these primers succeeded in finding all the TDG polymorphisms which, in different reports in literature, have missed either one or both of these polymorphisms.

Task 3: Months 7-12 Screening 100 research participants for polymorphisms in TDG

In our first screen of 100 ovarian cancer patients, we found that there are two high frequency polymorphisms for the TDG gene and no rare polymorphisms. The data is summarized in the following table. The observations were confirmed in all 100 cases both by CEL I mutation screening, and by Pyrosequencing that directly sequences the PCR amplified DNA for the base composition in each nucleotide position flanking the DNA polymorphism:

Blood genotype of first 100 patients

| Patient count | W+ | TDG exon 5 | TDG exon 10 | MED 1 exon 3 |
|---------------|-----|------------|-------------|--------------|
| 1 | | G/A | | G/A |
| 8 | | G/A | | |
| 1 | | A/A | | |
| 4 | | | G/A | G/A |
| 13 | | | G/A | |
| 1 | | | A/A | G/A |
| 1 | | | A/A | |
| 13 | | | | G/A |
| 58 | G/G | | | |

Total 100

| Allelic frequencies | | | Observed TDG exon 5 Ser + exon 10 Met | Expected TDG exon 5 Ser + exon 10 Met |
|-----------------------------|--------|-------|--|--|
| MED 1 exon 3 Ala --> Thr | 19/200 | 9.5% | | |
| TDG exon 5 Gly --> Ser | 12/200 | 6.0% | 0 | 3.0 |
| TDG exon 10 Val --> Met | 25/200 | 12.5% | 0 | 1.2 |

In this study, we learned that when the rare polymorphism frequency is below 0.005% (1/100 patients each with two potential alleles), there is insufficient statistical power to associate such polymorphisms with cancer even if we find a few of them in a large population of patients. In practical terms, few institutes have accomplished genotyping studies exceeding 2,000 patients because of the difficulty of the cost of analysis, patient recruitment, patient consent, and the need for detailed documentation of clinical history. As a result, we begin to focus only on the high frequency polymorphisms in our correlation of base excision repair gene polymorphisms with cancer.

As shown in above table, 58 patients have G/G in both alleles of the polymorphism position in MED 1 as well as the two polymorphic residues in TDG. The patients with MED1 polymorphism was randomly distributed among patients with or without either of the TDG polymorphisms, suggesting that the polymorphisms of these two related base excision repair genes have no apparent interactions. However, considering only the two polymorphisms of TDG in exon Ser and exon Met, they were unexpectedly excluding each other. Whereas 4.2 patients were expected to have one allele of exon 5 as Ser and one allele of exon 10 as Met, none were observed! This could be a statistical fluke, or a suggestion that such double allele patients do not live to adulthood, perhaps embryonic lethal. The implication is that the DNA polymorphisms are manifested in the gene

product of TDG and negatively influenced another essential function of TDG that is not its known role in DNA repair. We felt the project was ready for testing in breast cancer patients and proceeded to a breast cancer test panel of 168 patients.

Task 4: Months 7-12 Screening 168 breast cancer research participants for polymorphisms in TDG

We obtained the peripheral blood DNA of a panel of matched patients, with or without breast cancer, with or without BRCA1 mutation, about 43 patients in each of the four categories. We felt we should not engage a larger number of patients until a significant finding is apparent. We screened this panel with Pyrosequencing for the exon 5 and exon 10 polymorphism we found in the TDG gene followed by confirmation with CEL I mutation scanning. The results confirming the significance of our ovarian cancer panel study is shown in the following figure.

Allelic exclusion of exon 5 Ser and exon 10 Met polymorphisms in TDG gene

| Ovarian cancer | BRCA1 | breast cancer | exon 5 Gly --> Ser | | exon 10 Val --> Met | | Observed exon 5 Ser + exon 10 Met | Exon 5 Ser expected to have exon 10 Met | Exon 10 Met expected to have exon 5 Ser | Total patients exon 5 Ser + exon 10 Metn total |
|--|-------|---------------|--------------------|------|---------------------|-------|-----------------------------------|---|---|--|
| + | | | 12/200 | 6.0% | 25/200 | 12.5% | 0 | 3.0 | 1.2 | 4.2 |
| | +/- | + | 4/80 | 5.0% | 6/80 | 7.5% | 0 | 0.6 | 0.6 | |
| | +/- | - | 2/82 | 2.4% | 8/82 | 9.8% | 0 | 0.4 | 0.4 | |
| | +/+ | + | 2/88 | 2.3% | 7/88 | 8.0% | 0 | 0.3 | 0.3 | |
| | +/+ | - | 3/86 | 3.5% | 8/86 | 9.3% | 0 | 0.6 | 0.6 | |
| Breast study total | | | 11/336 | 3.3% | 29/336 | 8.6% | 0 | 1.9 | 1.9 | 3.8 |
| | | | | | | | | | | |
| Expected exon 5 Ser and exon 10 Met patients in both studies | | | | | | | | | | 8.0 |
| Observed exon 5 Ser and exon 10 Met patients in two studies | | | | | | | | | | 0 |

In this table, the ovarian cancer panel data is summarized in the first row. The data was obtained very recently and has yet to be subjected to statistical evaluation by our biostatistician collaborator Dr. Sam Litwin. The four breast cancer panels followed in rows 2-5. Although the patient number is still limited, it appears that the two TDG polymorphisms are unlinked to breast cancer, ovarian cancer, or the inheritance of the BRCA1 mutations. If this conclusion is correct, then the set of 268 patients can be synergistic in considering the mutual exclusion of the exon 5 and exon 10 polymorphisms in TDG. Indeed, where allelic frequencies of these polymorphisms would predict the presence of eight patients each harboring one allele of exon 5 Ser and one exon 10 Met, we observed none. The approximate statistical odd for such observation is about 1/256. More vigorous statistical analysis will follow. We believe the chance is apparently good that we have identified an important phenotype for TDG polymorphisms, and thus will proceed to strengthen the statistics further by planning to screen another 500 patients in the near future.

Plans to complete the proposed project in the extension period.

We have obtained approval to extend the project period by one year.

Task 5: Months 13-16 Screening 500 research participants for polymorphisms in TDG

We will screen for the exon 5 Ser and exon 10 Met polymorphisms in the DNA of 500 more patients.

Task 6: Months 13-25 Screening research participants for polymorphisms in OGG1

The second pair of BER enzymes to examine are OGG1 and hMYH. OGG1 is a DNA N-glycosylase that repairs the oxidation lesion of guanine by removing the 8-oxoguanine residue from an 8-oxo-G/A mismatch. hMYH is the human homolog of the E. coli MutY gene. It is a DNA N-glycosylase that removes the A residue that is misincorporated across an 8-oxo G residue present in the oxidized template strand. Some OGG1 polymorphisms are known to affect enzyme function.

We have collected the information on all known polymorphisms of the OGG1 gene that causes changes in the amino acid sequence.

| Codon | Position | DNA Alteration | AA Alteration |
|-------|----------|----------------|---------------|
| 46 | | G to A | Arg to Gln |
| 85 | 561 | G to T | Ala to Ser |
| 98 | | G to A | Lys to Lys |
| 131 | 730 | G to A | Arg to Gln |
| 154 | | G to A | Arg to His |
| 232 | 1032 | T to A | Ser to Thr |
| 308 | 1231 | G to A | Gly to Glu |
| 326 | 1285 | C to G | Ser to Cys |

We will identify these polymorphisms in the panel of 168 breast cancer patients used for the TDG study. We will establish their allelic frequencies, and test whether they have association with breast cancer, or with TDG polymorphisms. The latter is plausible because it is now appreciated that many DNA repair enzymes cluster at discrete loci in the nucleus during the repair reactions. In these loci, the repair enzymes may interact and some polymorphisms in their gene products may not be tolerated. If the correlations are found, we will extend the study to a larger panel of patients, such as the 500 new patients anticipated for the TDG study.

Task 7: Months 13-25 Screening research participants for polymorphisms in hMYH

We will identify these polymorphisms in the panel of 168 breast cancer patients used for the TDG study. We will establish their allelic frequencies, and test whether they have association with breast cancer, or with TDG polymorphisms.

KEY RESEARCH ACCOMPLISHMENTS:

1. The MED1 gene has only one known high frequency DNA polymorphism, and apparently no rare polymorphisms.
2. We confirmed that the TDG gene has only two known high frequency DNA polymorphisms and apparently no rare polymorphisms.
3. The two frequent polymorphisms appear not to be observed within the same person, suggesting that coexistence of both alleles may be embryonic lethal. The condition further suggests that the TDG gene has another role in early development independent of its known role in DNA repair.

REPORTABLE OUTCOMES:

None yet

CONCLUSIONS

The original hypothesis that having parallel pathways may lead to the accumulation of polymorphisms is certainly time tested to be true in bacteria, and may well be true in humans for some situations. In the case of the MED1 gene and the TDG gene, their apparently functional overlap has not led to detectable functional polymorphisms in the MED1 gene. It is not clear whether TDG polymorphisms, even as our data suggest that they have important new roles in the cell, arose because of functional overlap with the MED1 gene product. This possibility may be testable with future gene knockout experiments. Available data in this study does not support a role for MED1 and TDG polymorphisms, by themselves, as determinants of a significant fraction of breast cancer. Whether they may serve that role in combination with the polymorphisms of other base-excision repair genes will be revealed in future studies.

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APPENDICES

none